Appl. No. 10/522,037

Atty. Ref.: 3665-131 Supplemental Amendment

February 13, 2008

AMENDMENTS TO THE CLAIMS:

Amend the claims as follows:

Claims 1-23 cancelled.

24. (Currently Amended) A method of analysing a library of polynucleotides,

said polynucleotides being contained in cloning vectors having a particular host range,

the method comprising (i) selecting cloning vectors in the library which contain a

polynucleotide having a particular characteristic, (ii) modifying said selected cloning

vectors to allow a transfer and integration of said vectors and/or of the polynucleotide

which they contain into the genome a chromosome of a selected host cell, and (iii)

analysing the polynucleotides contained in said modified vectors upon transfer of said

modified vectors into said selected host cell.

25. (Previously Presented) The method of claim 24, wherein the library

comprises a plurality of unknown polynucleotides.

26. (Previously Presented) The method of claim 24, wherein the library

comprises a plurality of environmental DNA fragments.

27. (Previously Presented) The method of claim 24, wherein the cloning vectors

of the library are E. coli cloning vectors.

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28. (Previously Presented) The method of claim 24, wherein the selected

cloning vectors are modified by targeted insertion, into the vectors, of a target

polynucleotide construct.

29. (Previously Presented) The method of claim 28, wherein the targeted

insertion is performed in a region of the selected cloning vectors distinct from the

polynucleotide having a particular characteristic.

30. (Currently Amended) The method of claim 28, wherein the target

polynucleotide construct comprises [[an]]a functional origin of transfer functional-in the

selected host cell.

31. (Previously Presented) The method of claim 30, wherein the origin of

transfer is functional in E. coli host cells.

32. (Currently Amended) The method of claim 31, wherein the origin of transfer

is an origin of transfer contained in a plasmid selected from the group consisting of

RP4, pTiC58, F, RSF1010[[, ColE1]] and R6K(α).

33. (Previously Presented) The method of claim 28, wherein the target

polynucleotide construct comprises an integrase functional in the selected host cell.

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34. (Previously Presented) The method of claim 33, wherein the integrase is

35. (Previously Presented) The method of claim 29, wherein the target

polynucleotide construct comprises a transcriptional promoter functional in the selected

host cell.

36. (Previously Presented) The method of claim 28, wherein the target

polynucleotide construct comprises a transposable nucleic acid construct.

37. (Previously Presented) The method of claim 36, wherein the transposable

nucleic acid comprises, two inverted repeats, the target polynucleotide construct and a

marker gene, said inverted repeats flanking the target polynucleotide construct and the

marker gene.

38. (Previously Presented) The method of claim 24, wherein the cloning vector

comprises a first marker gene and wherein, in step ii), the cloning vector is modified by:

contacting in vitro, in the presence of a transposase, the selected cloning vectors

with a transposon comprising, two inverted repeats, the target polynucleotide construct

and a second marker gene distinct from the first marker gene with inverted repeats

flanking the target polynucleotide construct and the second marker gene, and

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selecting the cloning vectors which have acquired the second marker gene and

which have lost the first marker gene.

39. (Previously Presented) The method of claim 24, wherein, in step (i), the

cloning vectors which contain a polynucleotide having a particular characteristic are

selected by molecular screening.

40. (Previously Presented) The method of claim 24, wherein, in step (iii), the

modified cloning vectors are transferred into the selected host cell by conjugative

transfer.

41. (Previously Presented) The method of claim 24, wherein, in step (iii),

polynucleotides are analysed by determining the phenotype or properties of the host cell

upon transfer or expression of the modified vector.

42. (Previously Presented) A method for the identification or cloning of

polynucleotides encoding a selected phenotype, the method comprising (i) cloning

environmental DNA fragments into *E.coli* cloning vectors to produce a metagenomic

library, (ii) identifying or selecting cloning vectors in said library which contain DNA

fragments having a particular characteristic of interest, (iii) modifying the identified or

selected cloning vectors into shuttle or expression vectors for transfer and integration in

a selected host cell, (iv) transferring the modified cloning vectors into said selected host

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cell and (v) identifying or cloning the DNA fragments contained in said modified cloning

vectors which encode said selected phenotype in said selected host cell.

43. (Withdrawn) A transposable nucleic acid construct, wherein said construct

comprises an origin of transfer and elements for integration and selection in a selected

host cell genome flanked by two inverted repeats.

44. (Withdrawn) A library of polynucleotides, wherein said library comprises a

plurality of environmental DNA fragments cloned into cloning vectors, wherein said

environmental DNA fragments contain a common molecular characteristic and wherein

said cloning vectors are E. coli cloning vectors comprising a target polynucleotide

construct allowing transfer and integration of the environmental DNA into a selected

host cell distinct from E. coli.

45. (Withdrawn) A polynucleotide sequence comprising all or part of SEQ ID

NOs: 1 or 2, or of their complementary strand.

46. (Withdrawn) An oligonucleotide comprising SEQ ID NO: 3 or 4.

47. (Previously Presented) The method of claim 27, wherein the vectors are

selected from the group consisting of a cosmid, a fosmid, P1 and BAC vectors.

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